

# FINAL REPORT



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## Implementing technologies and strategies to maintain resistance to sunflower rust

### DAQ00073

#### Project Details

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#### Summary

This project proposes to implement resistance gene pyramiding strategies through close collaboration with Pacific Seeds. These strategies have been developed by Department of Primary Industries and Fisheries (DPI&F) researchers in two previous GRDC projects, DAQ356 and DAQ537. The gene pyramids will be incorporated into elite breeding material using techniques and technologies developed by DPI&F. These include the use of DNA markers.

If successful, a range of elite lines/commercial hybrids containing strategic resistance gene pyramids will be available to growers. These lines will provide the industry with a directed strategy to manage the sunflower rust pathogen and reduce the risk of outbreaks of the disease.

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## Conclusions

To our knowledge, the integration of pathotype analysis with molecular technologies to develop and implement a strategy to provide the industry with long-lasting resistance to a disease (as attempted to achieve in this project) has rarely been tested as a concept and almost never put into practice at a commercial level. There are numerous reports in the literature of DNA markers for resistance genes but there appears to have been little application of the technology at such an advanced breeding level.

Part of the reason for this is that most breeding programs do not have access to a comprehensive analysis of the pathogen and its evolution. Without this information, breeding for disease resistance to highly variable pathogens is more like an 'informed guess' than a science. It is the reason almost all breeding programs are on a 'treadmill' when it comes to disease resistance. The ability to gauge *a priori* whether a resistance will remain strong, robust and therefore long-lasting is not in our science repertoire. However, the integration of methods used in this project goes a long way towards providing a model that can be applied to predict which gene combinations are likely to extend the life of resistant hybrids.

The concept of strategically pyramiding resistance genes is particularly powerful in relation to hybrid crops, where a suite of parents containing resistance genes can be used to create hybrids containing multiple genes for resistance. This concept creates an ability to manoeuvre quickly if there is a pathotype shift by simply changing the combinations of parents containing resistance pyramids. In practice, a breeder would develop hybrids with different gene combinations as a matter of course and have a replacement ready when required. In this project we are at the stage where the breeder will take the completed elite pyramid lines and make a series of hybrid test crosses with a range of elite parental lines that contain additional resistance genes. The breeder will then identify those that perform best and produce data on a range of hybrids with high performance that may contain different resistance gene combinations. This will give the breeder the ability to quickly switch hybrids and resistances, if necessary.

The importance of understanding the pathogen in relation to disease management strategies is emphasised in this project. The identification and use of pathotypes is fundamental to other work including identifying and manipulating resistance genes to discovering markers for those genes and developing a pyramiding strategy. The possession and use of an extensive pathotype collection is the key to anyone seeking the 'holy grail' of durable resistance.

## Recommendations

An analysis of the diversity of pathotypes found in wild sunflower populations throughout Australia indicates that without a strategy to deploy resistance genes it will be difficult to maintain economic resistance among commercial hybrids. Seasonal conditions over the past decade have not been favourable for sunflower production, as such the problem of rust has been largely avoided. Breeders should continue to develop hybrids with rust resistance and, if possible, carry this out in a strategic manner, using either pyramids or multiple sources of resistance in hybrid production and with an understanding of the pathotypes present in the rust population.

## Outcomes

### Economic

Rust is regarded as the most important disease of sunflower in Australia. Under favourable conditions it is a major production constraint. During the 1970s and 1980s, when there was consistent annual production across the major growing regions in New South Wales (NSW) and Queensland (Qld), the rust pathogen established a complex population structure of pathotypes (races) in response to the use of hybrids containing single resistance genes. During this period, sunflower breeders were challenged to produce new resistant hybrids every two to five years in order to stay ahead of the changing rust population. Producing new hybrids is a costly process, and without a clear strategy to manage resistance genes, sunflower breeders soon found themselves on a treadmill as the pathogen quickly adapted to each new resistant hybrid.

The current project is a culmination of several successive projects in which the tools required to implement a strategy to extend the life of resistance genes were developed. These tools include the identification of new resistance genes

and the development of DNA markers that identify these genes. The markers provide methods for manipulating and combining resistance genes in a concise way, avoiding the labours and uncertainty of traditional breeding. Many plant breeders are under the misconception that simply combining resistance genes will provide durable resistance. This process will most likely not succeed unless the resistance genes are selected and combined (pyramided) in a strategic way. In fact, 'blindly' pyramiding resistance genes may increase pathotype complexity.

Strategically pyramiding resistance genes requires a detailed understanding of the pathogen and how it evolves. Genes that are to be pyramided need to be selected carefully to avoid the usual counter of pathotype selection. Hence, we identify specific genes for pyramiding based on an understanding of how the pathogen population has evolved and predicting what pathotypes it can or cannot produce in the future. This process is expected to result in longer-lasting resistance, however there is no guarantee that the pathogen will not find a way to evolve and overcome the resistance. There is only an expectation based on the probability that the likelihood is small.

The development of durable resistance has rarely been achieved because the tools required to implement the strategy have either been unavailable or not been applied to specific diseases. This project has established a process and methodology to combine knowledge with DNA technologies to enable strategic breeding for durable resistance; an outcome that previously was not a realistic breeding option. The project outputs have been a) the methods developed, b) the germplasm containing pyramided resistance genes and c) a greater understanding of the pathogen and its evolution. The outcome will be longer-lasting resistance and reduced costs across the industry.

## Achievement/Benefit

### Summary of Breeding Strategies for project DAQ073

#### Overview

A collaboration agreement was signed in 2005 with Pacific Seeds Pty Ltd to use its elite inbred lines as recipients of gene pyramids for rust resistance. The gene pyramid lines were developed by researchers from the Queensland Department of Primary Industries and Fisheries (DPI&F). Plant breeding resources and expertise to assist DPI&F researchers were provided by Pacific Seeds throughout the course of the project. A royalty agreement of 1% was negotiated for any commercial lines that contain resistance gene pyramids owned by DPI&F.

The breeding strategy was suggested in the initial project proposal and was designed to introgress pyramided resistance genes from the two lines R5R2 and P1P2 into elite female (34349) and male (24347) parental lines respectively. In practice, the strategy was altered to accommodate practical breeding considerations and in consultation with the sunflower breeder(s), with backcrossing limited to prevent recurrence of undesirable phenotypic characters in the available selections. This reduced population sizes needed for selection and alteration was achieved through the use of DNA markers and reaction phenotyping to determine the presence of the resistance gene pyramids in the progeny of each selfed generation. We were thus able to fix desirable agronomic phenotypes earlier in the process without repeated backcrossing.

Despite these changes we maintained the direction of the initial breeding proposal. Details of progress towards the breeding strategy of producing hybrids containing pyramided resistance genes are provided below. Further information on the development of additional pyramided lines and the identification of new pathotypes and new sources of resistance are also presented.

#### 1. Selected lines for pyramiding

##### **Pacific Seeds elite lines**

Two elite lines from the Pacific Seeds breeding program were selected to be recipients of the gene pyramid. They were chosen for their current rust resistance and agronomic qualities. Line 34349 is an elite inbred female and 24367 is an elite inbred male.

##### **DPI&F resistance gene pyramid lines**

Line P1P2 contains the genes P1 and P2; two repulsion-linked genes that cannot easily be fixed in a single line. They are separately susceptible to rust but have remained resistant in combination for more than 10 years. That is, no

pathotype with combined virulence to the P1 and P2 genes has ever been detected. However, the usefulness of P1 and P2 as a combination has been limited to F1 hybrids because their repulsion linkage means they could not be combined in a single breeding line. The DPI&F sunflower rust team therefore pursued an experimental program to recover coupling-linked P1 and P2 genes so they could be manipulated in a breeding program and introgressed into other lines.

The introgression of the P1P2 line with the Pacific Seeds elite male line has been robust. Because both P1 and P2 were originally derived from elite germplasm, the resulting selections have produced uniform and acceptable phenotypes. Seven of the top 18 selections are about to be test crossed with elite females by Pacific Seeds to determine their commercial potential. Note that this was the last step in the proposed breeding strategy but has not been completed within the life of the project due to circumstances previously mentioned.

Line R2R5. In this line the R2 gene is resistant to all pathotypes of rust in Australia while the R5 gene is susceptible to a limited number of pathotypes. No pathotype has been found that can attack both genes together so this combination is considered to be a good candidate for durable resistance. The R2 and R5 resistances are presumed to be single independent genes but some 'uncharacteristic' effects have been encountered with some crosses involving these lines, and 'erosion' of resistance can occur with successive generations of selection. The resistance of these lines is therefore likely to be more complex than reported and some difficulty with the introgression into elite germplasm was encountered. The development of this line is a generation behind that of P1P2 because the original pyramided parent had to be reselected after it was found that resistance was severely diminished in the F2 generation. The new selection has proved more robust in retaining its resistance. Pacific Seeds will continue to develop the line with guidance from the project team.

## 2. Use of DNA markers

Markers for resistance genes P1, P2, R5 and R2 were developed in previous projects and used, where possible, to select lines and individuals from each generation of the breeding cycle. We were mostly able to use the markers to direct selection but there was an occasional loss of the ability to detect one or more markers in resistant selections. We therefore modified our selection process by screening all generations with rust pathotypes as a double check against the DNA markers. In practice, we expect that DNA markers will not always provide a single means of selection where diverse sunflower germplasm is concerned. It is likely that resistance gene(s) at the same locus in the elite parental line 34349 contributed to the difficulty of selecting with the R5 and R2 DNA markers. In future we will test the parent receiving the pyramided genes for presence/absence of genes that could be at the same locus as the pyramid genes. Overall, the markers were essential for the development of the initial pyramided lines (P1P2 and R5R2) and helpful in guiding selection for the introgression of gene pyramids into elite commercial lines.

## 3. Use of resulting pyramided lines

Commercial sunflower hybrids are the result of a cross between elite male and female parents. Hence, after introgression, lines 34349 (female) and 24367 (male) could either be inter-crossed to produce a hybrid containing a four-gene pyramid or they could each be tested in hybrid combination with other elite male and female lines in the breeding program. If these 'other elite lines' also contain resistance genes the resulting F1 hybrids will contain a multiple gene pyramid, with the major component being the durable DPI&F resistance genes. It is likely this latter strategy will be used by Pacific Seeds to develop a suite of possible hybrid combinations with commercial potential.

This strategy allows the breeder to draw characteristics, including other resistance genes, from a wide range of elite germplasm while maintaining the strong link of the specific DPI&F gene pyramid. In this way the main pyramid can be protected from over-exposure by producing a large range of different hybrids, each containing a different combination of resistance genes. This type of strategy should be applied to breeding for resistance to all diseases that change rapidly and evolve new virulences and virulence combinations. The difficulty in achieving this is directly related to the breeding tools available and an understanding of 'if and how' the pathogen will adapt.

## 4. Additional pyramided lines

In addition to the two pyramided lines selected for the breeding collaboration with Pacific Seeds, a further 251 lines containing resistance-gene pyramids were produced using selected sources of resistance. Many of these pyramid lines were further advanced to create a) fixed lines containing multiple resistance genes and b) test-cross populations

to determine whether the genes combined in the pyramid were genetically linked. Linked genes would mean further development of the pyramid into a fixed line would be difficult, depending on the closeness of linkage. As such, a decision would need to be made as to whether the particular pyramid was worth the extra breeding effort. In the case of P1P2 we decided that the effort was justified.

The result of this work includes discovering the linkage relationships for numerous rust resistance genes; knowledge important in understanding which gene combinations can be achieved in future pyramiding work. This type of information is virtually unknown to plant breeders for any but the major diseases of the world's most important food crops.

Most, if not all, of the pyramided lines developed in this project will be offered to commercial seed companies under Material Transfer Agreements (MAT). However this material can be regarded only as germplasm and will require additional breeding for commercial agronomic qualities.

## 5. New Sources of Resistance

Twelve new sources of resistance were identified from imported germplasm and developed into fixed lines, some of which entered the pyramiding program described in 4 above.

Resistance was selected from 75 wild sunflower populations collected in the USA. Resistant selections have been introgressed with an elite commercial parent to create material ready for breeding. Wild germplasm often fails to maintain resistance during domestication and most breeders are reluctant to expend effort; tending instead to simply take what comes easily. As a consequence, a lot of good material is never used.

Our seed store contains some 500 accessions of seed collected from wild sunflower populations located throughout Australia. Many of these have been screened for resistance to rust and selections with resistance identified.

The rust-resistant material mentioned above will be released to commercial seed companies under Material Transfer Agreements.

## 6. Pathotype monitoring

Isolates of rust were collected regularly from cropping areas and wild sunflower populations located throughout Australia. The pathogen continues to display extraordinary diversity, with new pathotypes discovered in most collections. This monitoring is critical to 1) our identification and development of resistant germplasm, 2) our development of breeding strategies for gene pyramiding and 3) our ability to detect new virulent pathotypes and advise the industry of their likely impact on commercial hybrids.

A collection of more than 3,500 isolates spanning some 30 years is maintained by DPI&F. This collection represents an invaluable resource for present and future breeding for resistance to sunflower rust.

## Other Research

During the course of the project we were invited to accompany two scientists from the United States Department of Agriculture (USDA) on a trip to Western Australia and South Australia to collect wild sunflower seed from diverse habitats and locations. The trip (funded by the USDA) provided us with an opportunity to collect isolates of rust from several very remote locations and obtain seed from these populations for our wild-seed collection. Most of the remote sites where we collected rust yielded new pathotypes. Having this larger picture of pathotype diversity increases our ability to predict pathotype behaviour and advise sunflower breeders in relation to selection for resistance.

## Intellectual Property Summary

DPI&F entered into a collaboration agreement with Pacific Seeds for this project. A royalty payment "at the rate of one (1) percent per gene pyramid line contained in the said commercial line" was agreed upon.

## Additional Information

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Engel, B.C., Kong, G.A., Goulter, K.C., Aitken, E.A., Thompson, S.M., Mitchell, J.H.M., Kochman, J.K., Lawson, W., Shatte., Gulya, T. Diversity in the sunflower; *Puccinia helianthi* pathosystem in Australia. *Australasian Plant Pathology*, 35,657-670.

This paper (30/6/2008) is a review of all the GRDC/DPI&F-funded research undertaken since 1994.